GENERAL LIBRARY
JUL 27 1919
UNIV. OF MICH.

BIOLOGICAL BULLETIN

OF THE

Marine Biological Laboratory

WOODS HOLE, MASS.

VOL. XXXVI JUNE, 1919 No. 6

CONTENTS

		PAGE
THOMPSON, CAROLINE BURLING.	*The Development of the Castes of Nine Genera and Thirteen Species of Termites*	379
SEILER, J.	*Researches on the Sex-Chromosomes of Psychidæ (Lepidoptera)*	399
ALLEN, BENNET M.	*The Relation of the Pituitary and Thyroid Glands of Bufo and Rana to Iodine and Metamorphosis*	405
TANNREUTHER, GEORGE W.	*The Migration of Reproductive Organs from Parent to Buds in Hydra*	419
COLLETT, M. E.	*Resistance of Cilia to Cytolytic Agents*	424

PUBLISHED MONTHLY BY THE
MARINE BIOLOGICAL LABORATORY

PRINTED AND ISSUED BY
THE NEW ERA PRINTING COMPANY
LANCASTER, PA.

AGENT FOR GREAT BRITAIN
WILLIAM WESLEY & SON
28 Essex Street, Strand, London, W. C.

Single Numbers, 75 Cents. Per Volume (6 numbers), $3.00

Entered October 10, 1902, at Lancaster, Pa., as second-class matter under Act of Congress of July 16, 1894.

Editorial Staff

E. G. Conklin—*Princeton University.*

George T. Moore—*The Missouri Botanic Garden.*

T. H. Morgan—*Columbia University.*

W. M. Wheeler—*Harvard University.*

E. B. Wilson—*Columbia University.*

Managing Editor

Frank R. Lillie—*The University of Chicago.*

All communications and manuscripts should be sent to the Managing Editor. the University of Chicago, Sept. 15th to June 15th, or Woods Hole, Mass., June 15th to Sept. 15th. Subscriptions and other matter should be addressed to the Biological Bulletin, 41 North Queen Street, Lancaster, Pa

Vol. XXXVI. *June, 1919.* *No. 6.*

BIOLOGICAL BULLETIN

THE DEVELOPMENT OF THE CASTES OF NINE GENERA AND THIRTEEN SPECIES OF TERMITES.

CAROLINE BURLING THOMPSON,

DEPARTMENT OF ZOOLOGY, WELLESLEY COLLEGE.

In a former paper (Thompson, 1917), the writer showed that the newly hatched nymphs of the termite *Reticulitermes flavipes* Kol., 1.1 mm. long, although externally all alike, are differentiated by internal structural characters into two distinct types—(*a*) reproductive nymphs and (*b*) worker-soldier nymphs—from which develop (*a*) the three fertile adult castes, the reproductive forms, and (*b*) the two sterile adult castes, the workers and the soldiers. It was further shown that when the reproductive nymphs have attained a length of 1.3–1.4 mm. they are differentiated by internal characters into "nymphs of the first form" and "nymphs of the second form," which develop finally into two of the three adult reproductive castes;[1] at a much later period, when they have attained a length of about 3.75 mm., the worker-soldier nymphs become differentiated by internal characters into the worker and the soldier nymphs.

Bugnion (1912, '13) states that the soldier (nasutus) of *Eutermes lacustris*, a new termite species from Ceylon, is differentiated at the time of hatching, and may be distinguished from the other newly hatched nymphs by both external and internal structures, namely: the frontal process (corne frontale) and the large frontal gland.

These observations of Bugnion are not in accord with the work of Knower (1894) who states that the nasutus of *Eutermes rippertii* (?)—since identified by Mr. N. Banks as *E. pilifrons*

[1] The development of the third, wingless, reproductive caste of *R. flavipes* has not been worked out.

Vol. XXXVI. *June, 1919.* *No. 6.*

BIOLOGICAL BULLETIN

THE DEVELOPMENT OF THE CASTES OF NINE GENERA AND THIRTEEN SPECIES OF TERMITES.

CAROLINE BURLING THOMPSON,

DEPARTMENT OF ZOOLOGY, WELLESLEY COLLEGE.

In a former paper (Thompson, 1917), the writer showed that the newly hatched nymphs of the termite *Reticulitermes flavipes* Kol., 1.1 mm. long, although externally all alike, are differentiated by internal structural characters into two distinct types—(*a*) reproductive nymphs and (*b*) worker-soldier nymphs—from which develop (*a*) the three fertile adult castes, the reproductive forms, and (*b*) the two sterile adult castes, the workers and the soldiers. It was further shown that when the reproductive nymphs have attained a length of 1.3–1.4 mm. they are differentiated by internal characters into "nymphs of the first form" and "nymphs of the second form," which develop finally into two of the three adult reproductive castes;[1] at a much later period, when they have attained a length of about 3.75 mm., the worker-soldier nymphs become differentiated by internal characters into the worker and the soldier nymphs.

Bugnion (1912, '13) states that the soldier (nasutus) of *Eutermes lacustris*, a new termite species from Ceylon, is differentiated at the time of hatching, and may be distinguished from the other newly hatched nymphs by both external and internal structures, namely: the frontal process (corne frontale) and the large frontal gland.

These observations of Bugnion are not in accord with the work of Knower (1894) who states that the nasutus of *Eutermes rippertii* (?)—since identified by Mr. N. Banks as *E. pilifrons*

[1] The development of the third, wingless, reproductive caste of *R. flavipes* has not been worked out.

Holmgren—arises late in its development by molting from an antecedent worker-like form with thirteen antennal segments.

In view of these conflicting observations it seemed at first possible that different degrees of differentiation of the newly hatched nymphs, as well as differences in development, might exist in the various genera of termites, and perhaps even among the species of a genus, as, for example, in the genus *Eutermes;* the nasutus of *E. lacustris* being clearly defined at the time of hatching, according to Bugnion; the nasutus of *E. pilifrons* arising rather late in the development by molting from a worker-like form, according to Knower. But, before coming to any conclusion, it seemed desirable to have further data in regard to the conditions among other termites at the time of hatching and in the early stages of development.

The present study of the newly hatched and the developing nymphs of nine genera and thirteen species of American termites, from both temperate and tropical regions, was therefore undertaken for two reasons: first, to ascertain the conditions among different genera of termites, and second, to determine whether the development may vary within a genus, by comparing the conditions in *Eutermes lacustris*, as described by Bugnion, with those of *Eutermes pilifrons*, the Jamaican species observed by Knower, and with two other tropical species, *E. morio* Latreille, and *E. sanchezi* Holmgren.

Material.

Dr. Knower has very kindly sent me an abundance of preserved material of *Eutermes pilifrons*, consisting of eggs, young nymphs, winged adult reproductive forms, and adult workers and nasuti. I am indebted to Mr. N. Banks, of the Museum of Comparative Zoölogy, Cambridge, Mass., for the identification of this species.

Most of my material was furnished me by the Bureau of Entomology of the U. S. Department of Agriculture, and much of it was collected and fixed for me by Mr. T. E. Snyder, to whom my sincere thanks are due. The eggs and young of *Termopsis* I have collected myself at Pacific Grove, Cal., during a visit to the Hopkins Marine Station.

Gilson's fluid has proved a good fixative for the newly hatched nymphs, and Bouin's fluid is excellent for all older forms. As in the case of *Reticulitermes* whole mounts of the newly hatched and developing nymphs, stained with Conklin's picro-hæmatoxylin, are most useful for preliminary study.

The termites to be described in this paper are *Termopsis angusticollis* Walker, *Calotermes* n. sp. Banks, *Cryptotermes cavifrons* Banks, *Neotermes castaneus* Burm., *Arrhinotermes simplex* Hagen, *Reticulitermes flavipes* Kollar, *R. virginicus* Banks, and *R.* n. sp. Banks, *Anoplotermes fumosus* Müller, *Amitermes tubiformans* Buckley, *Eutermes pilifrons* Holmgren, *E. morio* Latreille and *E. sanchezi* Holmgren.

Family.	Genus and Species.	Length, Egg, Mm.	Length, Newly-hatched Nymphs, Mm.	Length, Small and Large Headed Nymphs, Mm.	No. Antennal Segments, Newly Hatched Nymphs.
Protermitidæ . .	*Termopsis angusticollis*. . . .	1.3 –1.7	?	2.8–3	?
	Calotermes n. sp. .	1.2 –1.4	1.6	1.8–2	9, third entire.
	Cryptotermes cavifrons.	1.2 –1.3	1.4 –1.6	2.4	9, third entire.
	Neotermes castaneus.	1.6	?	2.6	?, probably 9.
Mesotermitidæ.	*Arrhinotermes simplex*.	0.8 –1	1.2	2	9, third subdivided.
	Reticulitermes flavipes.	0.68–0.7	1.1	2	9, third subdivided.
Metatermitidæ.	*Anoplotermes fumosus*.	0.56–0.6	?	1.8–2	?, probably 11.
	Amitermes tubiformans. . . .	0.56–0.64	0.95–1.0	1.6–1.8	11, third subdivided.
	Eutermes pilifrons	0.64–0.7	0.8 –0.9	1.8–2	11, third subdivided.

In the accompanying table it may be seen that the first four genera belong to the family Protermitidæ of Holmgren, the fourth, together with *Reticulitermes*, belongs to the Mesotermitidæ, and the last three are Metatermitidæ. The present paper therefore includes examples from each family of the order Isoptera.

My study includes for each genus (1) the eggs, (2) the newly hatched nymphs, in some cases these have been actually dis-

sected out from the egg shell, in other cases they are slightly older; (3) an early stage of development, usually 0.2–0.4 mm. longer than the newly hatched nymphs, in which the external appearance, and especially the size of the heads, is similar in all nymphs; (4) a slightly older stage, varying considerably in length in the different genera, in which an external differentiation may be noted in the size of the heads, dividing the developing nymphs into "small headed" (reproductive) forms, and "large headed" (sterile) forms.

PROTERMITIDÆ.

Calotermes n. sp. Banks.—The eggs of the most primitive termites, those belonging to the family Protermitidæ, are the largest of the order Isoptera.

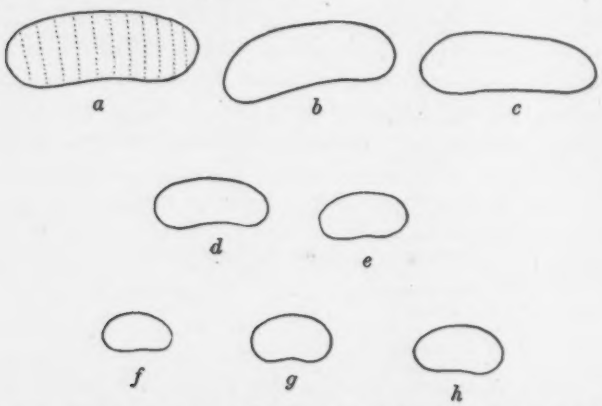


FIG. 1. Termite eggs. *a, Termopsis angusticollis; b, Calotermes* n. sp.; *c, Cryptotermes cavifrons; d, Arrhinotermes simplex; e, Reticulitermes flavipes; f, Anoplotermes fumosus; g, Amitermes tubiformans; h, Eutermes pilifrons.* Spencer Oc. 6, obj. 32, reduced one third.

The eggs of *Calotermes* n. sp. (Fig. 1, *b*) are long, slender, and reniform, and are slightly larger at one end, the future head end; they measure, in preserved specimens, from 1.2–1.4 mm. in length. In all the species of termites here described the egg length varies several tenths of a millimeter. It would be interesting to know whether these variations in length are constant in the living eggs, and whether they represent two different sizes

of eggs, corresponding to the two different types of nymphs which are hatched. Since this paragraph was written further study of living eggs enables me to state with certainty that there are marked differences in size in the living eggs of a given species and, furthermore, among eggs in the same phases of development.

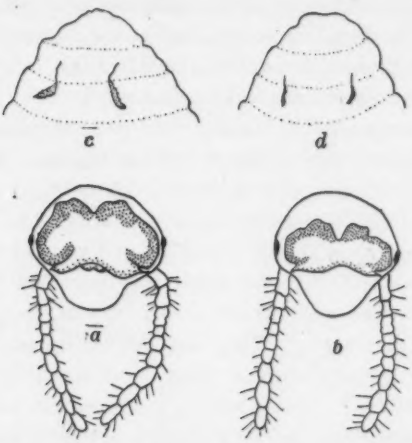


FIG. 2. *Calotermes* n. sp., newly hatched nymphs. *a*, head of reproductive nymph with large brain; *b*, head of soldier nymph with small brain; *c*, testes, reproductive nymph; *d*, testes, soldier nymph. Oc. 6, obj. 16, reduced one third.

The newly hatched nymphs of *Calotermes* n. sp. are 1.6 mm. long, with nine antennal segments, the third segment entire and bare. In size, shape and general external appearance these newly hatched nymphs are all alike, but, as in the genus *Reticulitermes*, they are separable by means of internal structures into two types: (*a*) nymphs with a large brain that almost fills the cavity of the head, large sex organs, and a white dense abdomen, the reproductive or fertile type (Fig. 2, *a*, *c*); and (*b*) nymphs with a smaller brain that does not nearly fill the head cavity, smaller sex organs, and a transparent abdomen, the soldier,[1] or sterile type (Fig. 2, *b*, *d*).

When the *Calotermes* nymphs have attained a body length of 1.8–2 mm. a marked difference in the size of the heads is noted,

[1] The worker caste is lacking in the genus *Calotermes*.

the fertile, or reproductive type having a small head with a larger brain, the "small-headed" nymph of Grassi (1896–97); the sterile, or soldier type, with larger head and smaller brain, the "large-headed" nymph of Grassi. The number of antennal segments in these older nymphs is still nine, although the third segment is deeply grooved and subdivided into two parts. The increase in the number of antennal segments is therefore slightly slower than in the genus *Reticulitermes*, the "small-headed" and "large-headed" nymphs of *Reticulitermes*, 2 mm. in length, having twelve clearly defined antennal segments.

Termopsis angusticollis Walker.—*Termopsis angusticollis* produces the largest egg of any American termite. In form, the eggs are long, slender, and reniform, slightly larger at the future head end, and sometimes with surface markings (Fig. 1, *a*). Both in the living eggs and after fixation, and the eggs shrink very little in Gilson's Fluid, two fairly distinct sizes of eggs may be observed. The smallest are about 1.3 mm. long, the largest range from 1.5–1.7 mm. These size differences are independent of embryonic growth, since a large and a small egg may be in the same phase of development. I am unable to state what types of nymphs may hatch from the different sized eggs, for after searching repeatedly in many colonies in Pacific Grove, Cal., I do not feel sure that I have seen the newly hatched nymphs. The smallest nymphs that I have found are 2.2 and 2.5 mm. long, with eleven and twelve antennal segments. These may be the newly hatched forms, but their size in relation to the egg, their activity, for newly hatched nymphs are usually sluggish, as well as the eleven or twelve antennal segments, inclines me to believe that they have been hatched for a short time. In one embryo with well formed appendages nine segments could be distinguished in the antennae. I am therefore unable to make any definite statement regarding the body length and the number of antennal segments of the newly hatched nymphs of *Termopsis angusticollis*, although from the size of the egg I should expect the nymphs to measure about 1.6–1.9 mm. in length, and the number of antennal segments to be like that of the other Protermitidæ, *Calotermes* and *Cryptotermes*, namely, nine, with the third segment either entire or subdivided.

The youngest nymphs of *T. angusticollis* that I have examined —2.2–2.5 mm. long, with eleven and twelve antennal segments —are all alike in external macroscopic appearance, but with a lens the two types of fertile or reproductive nymphs and sterile or soldier[1] nymphs may be seen. The heads are of similar size, but the large brain almost filling the head cavity of the reproductive nymph (Fig. 3, *a*) is clearly distinguished from the smaller brain of the soldier nymphs (Fig. 3, *b*), and correlated with the brain structure is the whiter denser abdomen of the reproductive nymphs and the more transparent abdomen of the soldier nymphs. In stained and mounted specimens the larger sex organs of the reproductive nymphs are in marked

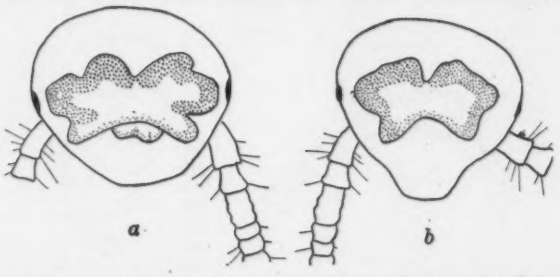


FIG. 3. *Termopsis angusticollis*, young nymphs 1.8 mm. long, with twelve antennal segments. *a*, head of reproductive nymph with large brain; *b*, head of soldier nymph with small brain. Oc. 6, obj. 16, reduced one third.

contrast to the smaller ones of the soldier nymphs. The brain of all the adult castes of *T. angusticollis* has a very characteristic form, as if it had been pulled out laterally, due to the lateral extension of the optic lobes which extend out at right angles to the long axis of the body. This peculiar form of the brain is recognizable even in the youngest nymphs.

Slightly older nymphs of *Termopsis*—2.8–3 mm., with thirteen and fourteen antennal segments—have the heads differentiated in size so that the "small-headed," large-brained, reproductive forms are easily distinguishable from the "large-headed," small-brained, soldier nymphs.

Cryptotermes cavifrons Banks.—The egg of *Cryptotermes*, like

[1] The worker caste is lacking in the genus *Termopsis*.

those of the other Protermitidæ here described, is long slender and reniform, and measures 1.2–1.3 mm. in length (Fig. 1, *c*).

The newly hatched nymphs are 1.4–1.6 mm. long and have nine antennal segments, the third segment entire and bare. These nymphs are externally all alike, but are differentiated internally into the reproductive type with large brain, and large sex organs (Fig. 4, *a*), and the soldier[1] type with smaller brain and smaller sex organs (Fig. 4, *b*).

The peculiar truncated head and the characteristic form of the antennae of this species are recognizable, even in the newly hatched nymphs.

In nymphs which are about 2.4 mm. long the "small-headed" and "large-headed" types are clearly seen. This termite, like

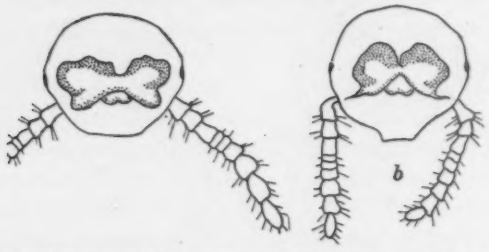


FIG. 4. *Cryptotermes cavifrons*, newly hatched nymphs. *A*, head of reproductive nymph with large brain; *b*, head of soldier nymph with small brain. Oc. 6, obj. 16, reduced one third.

Calotermes n. sp., increases the antennal segments slowly, for nine segments still occur in this older phase, although the third segment is subdivided.

Neotermes castaneus Burmeister.—The egg of *Neotermes*, fig. 5, *c*, is large, measuring 1.6 mm. in length. In form it is slender and reniform like the eggs of other Protermitidae.

The youngest nymphs in my material, which is not very abundant, are 2 mm. long and have ten antennal segments. Since the majority of the termites here described have nine antennal segments when hatched and are only from 0.2–0.3 mm. longer than the egg from which they have emerged, I believe that these nymphs are very slightly past the age of hatching. An examination of the antennae gives further basis for this opinion, fig.

[1] The worker caste is lacking in the genus *Cryptotermes*.

5, *a*, *b*. The fourth antennal segment, which in growing individuals is always the youngest, is short and bears very minute hairs, and in one specimen bears hairs on one side only, indications that this segment has been very recently cut off from the third, probably since the time of hatching.

Externally these young nymphs of *Neotermes* are still all alike, for no differentiation in the size of the heads can be observed, but internally they are differentiated into the two types of nymph

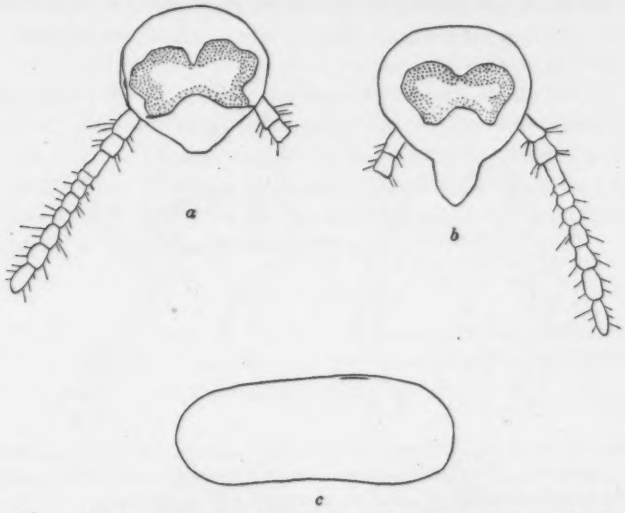


FIG. 5. *Neotermes castaneus*, *a*, head of young reproductive nymph, 1.8 mm. long; *b*, head of young soldier nymph, 1.8 mm. long; *c*, egg. *a*, *b*, Leitz oc. 1, obj. 3; *c*, Leitz oc. 4, obj. 1; reduced one third.

found in the other Protermitidae, namely: the reproductive or fertile type, with large brain, and large sex organs, and the soldier or sterile type, with small brain and small sex organs. The heads of these two types are shown in fig. 5, *a*, *b*.

Nymphs 2.6 mm. in length and with eleven antennal segments have the heads differentiated into the small-headed reproductive type with large brain, and the large-headed soldier type with small brain.

All four genera of Protermitidae are therefore alike in possessing two types of nymphs at the time of hatching.

MESOTERMITIDÆ.

Arrhinotermes simplex Hagen.—The eggs of the Mesotermitidæ are considerably smaller than those of the Protermitidæ. The eggs of *Arrhinotermes simplex* measure from 0.8 to 1 mm. in length and are slender and reniform (Fig. 1, *d*).

The newly hatched nymphs are about 1.2 mm. long, and have nine antennal segments, the third segment grooved and subdivided into two parts.

The two types of nymphs: (*a*) reproductive nymphs, with large brain and large sex organs (Fig. 6, *a*, *c*), and (*b*) worker-soldier

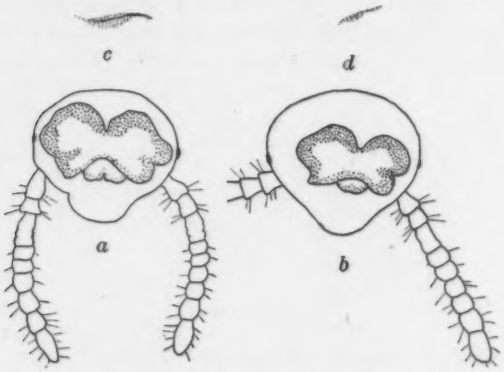


FIG. 6. *Arrhinotermes simplex*, newly hatched nymphs. *a*, head of reproductive nymph; *b*, head of worker-soldier nymph; *c*, ovary of reproductive nymph; *d*, ovary of worker-soldier nymph. Oc. 6, obj. 16, reduced one third.

nymphs, with small brain and small sex organs (Fig. 6, *b*, *d*), are likewise present in this genus at the time of hatching; and with an increase of the body length to 2 mm., the "small-headed" and "large-headed" nymphs are differentiated. There is a striking resemblance between the young of this genus and the related genus *Reticulitermes*.

Reticulitermes.

R. flavipes Kollar.[1] The egg measures from 0.68 to 0.7 mm. (Fig. 1, *e*). The newly hatched nymphs are 1.1 mm. long, with nine antennal segments, the third segment grooved, and are of

[1] For a fuller description of the development of *R. flavipes*, see Thompson (1917).

the two types, reproductive nymphs and worker-soldier nymphs. Nymphs 2 mm. long with twelve antennal segments are differentiated into "small-headed" and "large-headed" types.

R. virginicus Banks.—The newly hatched nymphs of *R. virginicus* are 1 mm. long and have nine antennal segments, the third grooved. They are differentiated into the two types of large-brained reproductive forms and small-brained sterile forms. The further development of this species, so far as I have followed it, is very similar to and probably identical with that of *R. flavipes*.

R. n. sp. Banks.—The young nymphs of a new species of *Reticulitermes* not yet described have been given me by Mr. T. E. Snyder. Examination of this species shows that the conditions at the time of hatching are similar to those in *R. flavipes* and in *R. virginicus*. It seems more than probable therefore that this differentiation of the newly hatched nymphs into two types is common to all species of *Reticulitermes*.

METATERMITIDÆ.

Anoplotermes fumosus Müller.—The eggs of the Metatermitidæ are smaller than either of the other families of termites. The eggs of *A. fumosus* measure from 0.56 to 0.60 mm. in length (Fig. 1, *f*).

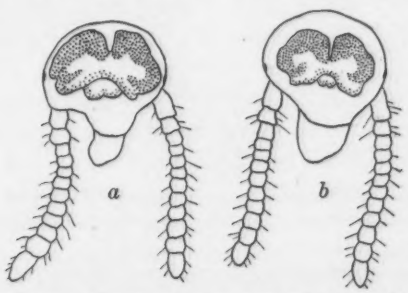


FIG. 7. *Anoplotermes fumosus*, young nymphs 1 mm. long, with twelve antennal segments. *a*, head of reproductive nymph; *b*, head of worker-soldier nymph. Oc. 6, obj. 16, reduced one third.

The youngest specimens of *Anoplotermes fumosus* that I have examined are in an early phase of development, but, on account of their size and the number of antennal segments, I do not think

that they are the newly hatched forms. These specimens measure from 1 to 1.2 mm. in length, and have twelve antennal segments, the third segment being deeply grooved or subdivided. Like the very young nymphs of the other termite genera described above, these young nymphs of *A. fumosus* are externally all alike but are differentiated internally into (*a*) the reproductive nymphs, with large brain and large sex organs (Fig. 7, *a*), and (*b*) the worker-soldier nymphs, with small brain and small sex organs (Fig. 7, *b*). An unusually long slender labrum is present in both types of nymphs, and is characteristic of the three genera of this family here described.

The older nymphs of *A. fumosus*, about 2 mm. long, or less, are differentiated into the "small-headed," large-brained, reproductive type, and the "large-headed," small-brained worker-soldier type. My material was not very abundant, but was sufficient to show that the early development of *A. fumosus* is similar to that of the other termites here described.

Amitermes tubiformans Buckley.—The eggs of *A. tubiformans* measure from 0.56 to 0.64 mm. in length (Fig. 1, *g*).

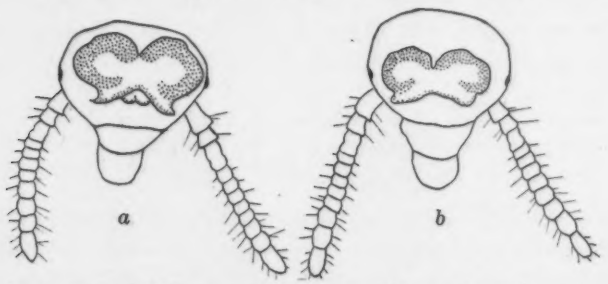


FIG. 8. *Amitermes tubiformans*, newly hatched nymphs. *a*, head of reproductive nymph; *b*, head of worker nymph. Oc. 6, obj. 16, reduced one third.

The youngest nymphs examined are from 0.95 to 1. mm. long, and have antennæ with eleven segments, the third segment bare, but subdivided into two parts. One of these nymphs had a piece of the egg shell still adhering to the abdomen, so that they are evidently the newly hatched forms.

The newly hatched nymphs of *A. tubiformans* are externally all alike, but are differentiated internally into (*a*) reproductive

nymphs with large brain and large sex organs (Fig. 8, *a*), and (*b*) worker[1] nymphs, with small brain and small sex organs (Fig. 8, *b*). The long labrum is prominent in the nymphs of both types.

Nymphs 1.6 –1.8mm. long, with twelve antennal segments, are differentiated into the "small-headed" and "large-headed" types.

Eutermes.

E. morio Latreille.—The eggs of this species of *Eutermes* measure from 0.68 to 0.72 mm. in length and are slender and reniform. The newly hatched nymphs are 1–1.2 mm. long; they have eleven antennal segments, and they consist of the large-brained reproductive type and the small-brained worker-soldier type.

E. sanchezi Holmgren.—I have not seen the eggs of this species but the newly hatched nymphs are very similar to those of *E. morio*. They are 1.2 mm. long, with eleven antennal segments, and the two types of reproductive and worker-soldier nymphs are present.

The newly hatched nymphs of *E. morio* and *E. sanchezi* are almost identical in structure with those of *E. pilifrons* and are therefore not figured; their later development has not been studied, but there is no reason to believe that the three species, which are alike in their early development, should differ in the later phases.

E. pilifrons Holmgren.—The eggs of *E. pilifrons* measure from 0.64 to 0.72 mm., and are slender and reniform (Fig. 1, *h*).

This species was formerly described as *E. rippertii* (?) by Knower, and I am indebted to Dr. Knower for my very abundant material. Many of the nymphs have actually been dissected out from their egg shells, so that there is absolutely no uncertainty as to the structure or the size of the newly hatched forms.

The newly hatched nymphs of *E. pilifrons* measure from 0.8 to 0.9 mm., the difference in length being due to whether the nymphs are still in the curved embryonic position or have straightened out. The number of antennal segments is eleven,

[1] The soldier caste is lacking in the genus *Amitermes*.

with the third segment grooved and subdivided into two parts, so that twelve and thirteen segments will be formed soon after hatching. It is interesting to note the progressive development of the termite order in as small a detail as the number of segments in the antennæ at the time of hatching. The Protermitidæ have nine antennal segments, with the third segment entire; in the intermediate Mesotermitidæ the number is still nine, but the third segment is grooved or subdivided into two parts; in the most highly developed Metatermitidæ the number has increased to eleven, with the third segment subdivided.

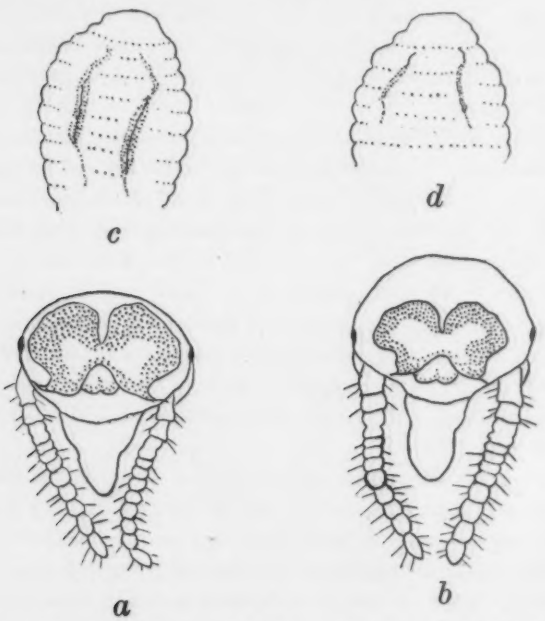


FIG. 9. *Eutermes pilifrons*, newly hatched nymphs. *a*, head of reproductive nymph; *b*, head of worker-soldier nymph; *c*, ovaries of reproductive nymph; *d*, ovaries of worker-soldier nymph. Oc. 6, obj. 16, reduced one third.

E. pilifrons, like all the other termites described in this paper, has the two types of newly hatched nymphs which are alike in external structure:—the reproductive nymphs, with a large brain and large sex organs (Fig. 9, *a*, *c*), and the worker-soldier

nymphs, with a smaller brain and smaller sex organs (Fig. 9, *b*, *d*). The difference between the bodies of fixed specimens of the two types is more marked in this termite than in any other that I have examined; the body of the sterile worker-soldier individuals is a very clear and transparent glistening white, while that of the reproductive individuals is a dull opaque creamy white. A long slender labrum is noticeable in all nymphs of both types.

No appreciable difference in the size of the heads is noted until the nymphs have attained a length of about 2 mm., and have twelve antennal segments. In this phase the "small-headed" reproductive forms with large brain and creamy white body are easily distinguishable with a hand lens from the "large-headed" worker-soldier forms with small brain and transparent glistening white body.

In worker-soldier individuals about 2 mm. long and with twelve antennal segments there is as yet no external differentiation between the two sterile castes, but an internal differentiation has already begun and may be observed in whole mounts of stained individuals as well as in sections. The future soldiers are distinguishable by the presence of the larger frontal gland which appears, in frontal mounts of the head, as a small dense spot posterior to the brain; in whole mounts of the head of the future worker no such spot is visible. After examining the stained specimens in cedar oil to separate the future soldiers from the future workers, the two kinds of individuals were embedded and sectioned. In the soldier nymphs a large, although embryonic, frontal gland opens to the exterior on the frontal surface of the head. This gland was more than three times the size of the vestigial gland found in the worker nymphs.

The soldier caste of *E. pilifrons* is, therefore, not differentiated by external characters at the time of hatching, but arises later in the development, being first manifested in individuals about 2 mm. long with twelve antennal segments. The worker caste is differentiated at the same time, and the two castes may be recognized by the size and structure of the respective frontal glands; although no external differentiations are yet present in either caste.

The differentiation of the worker-soldier nymphs of *E. pilifrons* into the worker and the soldier is nearly parallel with the development of these two castes from the worker-soldier form in the genus *Reticulitermes*, Thompson (1917, pp. 123–125); the chief difference being the age of the respective nymphs, the differentiation being visible in *E. pilifrons* in nymphs 2 mm. long, while in *R. flavipes* it was first observed in nymphs 3.75 mm. long, although, from the maturity of the frontal gland, it could probably be seen in an earlier phase.

My work upon this species of *Eutermes* is in perfect accord with the work of Knower (1894), who noted the absence of young soldier nymphs in the colonies of *E. rippertii* (?) = *E. pilifrons* Holm., and who later saw a young soldier of this species emerge by molting from a worker-like skin. The mandibles on the skin were large, as in *Eutermes* workers, while those of the emerging soldier (nasutus) were small, like those of the adult nasuti. The head of this nasutus was light in color and not yet fully elongated.[1]

Bugnion (1912) states that the soldier (nasutus) of *E. lacustris* is differentiated at the time of its emergence from the egg, and is distinguished from the other newly hatched nymphs by a long frontal process or horn, and a frontal gland with an excretory canal. His description is as follows:

"Larve de soldat, longue de 1.32 mm, venant d'éclore (Fig. 12). Cette forme est particulièrement intéressante parce qu'elle montre une petite corne implantée au dessus du front. Elle donne ainsi la preuve que la caste 'soldat' se différencie déjà dans l'oeuf. La corne, tres courte, ne dépasse pas le niveau des pièces buccales. On voit aussi, par transparence, l'ampoule céphalique entourée de muscles et, à la base de la corne, le canal excréteur."

Now, although I am fully in accord with the view of Bugnion that the castes of termites are not produced by food or other external influences, and that they are due to intrinsic deep-

[1] Snyder (1915) has also described the origin of a soldier nymph from a worker-like form in *Reticulitermes flavipes* and *R. virginicus*. The nymphs were found in the quiescent stage that precedes a molt, and the emergence of the young soldiers was observed. In this case, too, the mandibles of the emerging form differed from those on the cast skin.

seated causes, I am inclined to believe, for several reasons which follow, that Bugnion's account of the newly hatched soldier (nasutus) nymph of *E. lacustris* may be capable of a different interpretation.

First, the conditions among the newly hatched and the younger developing phases of the nine termite genera here described are practically similar, showing that throughout the entire order of termites the early development is remarkably constant.

Second, I have examined the newly hatched and the developing nymphs of three species of *Reticulitermes*—*R. flavipes*, *R. virginicus* and *R.* n. sp.—and also of three species of *Eutermes*—*E. pilifrons*, *E. morio* and *E. sanchezi*—and in each case the development of the three species is similar throughout the early phases and as far as I have followed it. It seems probable, therefore, that *E. lacustris* would follow the plan of development that is common not only to three other species of its genus and to three species of *Reticulitermes*, but to seven other genera.

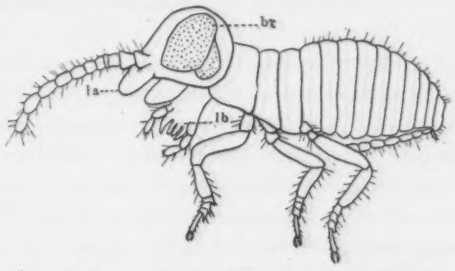


FIG. 10. *Eutermes pilifrons*, newly hatched nymph, profile view. *la*, labrum; *lb*, labium; *br*, brain. Oc. 6, obj. 16, reduced one half.

Third, in all the newly hatched nymphs of *Eutermes*, whether *pilifrons*, *morio* or *sanchezi*, the labrum, as stated above, is long slender and conspicuous. In nymphs seen in profile the side view of this labrum is surprisingly like the frontal process or "corne frontale" of a nasutus. In studying the younger *Eutermes* nymphs in alcohol I remarked again and again the length of the labrum and its resemblance, especially in profile view, to the frontal process of a nasutus. This is also true of the other two genera of the Metatermitidæ here described, *Anoplotermes* and *Amitermes*.

In my Fig. 10, the profile view of a recently hatched *worker-soldier* nymph of *E. pilifrons*, the resemblance is very striking to Bugnion's figure 12 (1912), the profile view of the form which Bugnion has described as a newly hatched *soldier* nymph of *E. lacustris*. The long labrum, *la*, might have been taken for the "corne frontale," the dark stippled mass, *br*, which represents the brain as seen through the transparent skin of the head, might easily be mistaken for the frontal gland that is shown in Bugnion's figure in about the same position. The duct of the frontal gland figured by Bugnion might prove to be the esophagus, which passes forward as a slender tube beneath the brain. Furthermore, the frontal gland as figured by Bugnion in the newly hatched soldier (nasutus) of *E. lacustris* is much larger and more highly developed than in the older nymphs of *E. pilifrons* 2 mm. long, and is also larger and more developed than I have found it in any newly hatched termite nymphs that I have studied. As a rule this gland is not distinguishable at the time of hatching except in sections, and even then is extremely small and difficult to determine.

Fourth, Professor Bugnion's figure was drawn from an unstained specimen, mounted, as he states, in either water or weak formol, and studied as a transparent object. If after staining and sectioning the same results are obtained in the newly hatched nymphs of *E. lacustris* we should then have positive proof that development varies in the different species of a termite genus and that some castes may be fully differentiated at the time of hatching. But, until this proof is established, the writer is inclined to believe that development is a constant process among the different species of termites, and that it takes place as in the termites here described.

Summary.

The termites described in this paper are:

Protermitidæ—

Termopsis angusticollis.
Calotermes n. sp.
Cryptotermes cavifrons.
Neotermes castaneus.

MESOTERMITIDÆ—

Arrhinotermes simplex.
Reticulitermes flavipes.
" *virginicus.*
" n. sp.

METATERMITIDÆ—

Anoplotermes fumosus.
Amitermes tubiformans.
Eutermes morio.
" *sanchezi*
" *pilifrons.*

1. The eggs of these termites are largest in the primitive forms, the Protermitidæ, measuring from 1.2 to 1.7 mm. in length, they are smaller in the Mesotermitidæ, from 0.68 to 1 mm. long; and are smallest in the Metatermitidæ, from 0.56 to 0.72 mm. long.

2. The newly hatched nymphs correspond in size to the eggs from which they have hatched.

3. The newly hatched nymphs are externally all alike, but they are differentiated by internal structural characters into *two* clearly defined types: (*a*) the *reproductive*, or *fertile forms*, with large brain and large sex organs, and usually a dense opaque body; and (*b*) the *worker-soldier*, or *sterile forms*, with small brain and small sex organs, and usually a clear transparent body.

4. At the time of hatching the antennæ of the Protermitidæ (*Calotermes* n. sp., *Cryptotermes cavifrons*, and probably also *Termopsis angusticollis* and *Neotermes castaneus*) have nine segments, with the third segment entire; those of the Mesotermitidæ (*Arrhinotermes simplex*, *Reticulitermes flavipes*, *R. virginicus* and *R.*, n. sp.) have nine segments with the third subdivided; those of the Metatermitidæ (*Amitermes tubiformans*, *Eutermes pilifrons*, *E. morio* and *E. sanchezi*, and probably also *Anoplotermes fumosus*) have eleven segments, the third subdivided.

5. Nymphs with a body length of about 2 mm., or 2.5 to 3 mm. in the larger genera, are differentiated into "small-headed" but large brained reproductive forms, and "large-headed" but small-brained worker-soldier forms.

6. The worker-soldier nymphs of *Eutermes pilifrons*, 2 mm.

long, with twelve antennal segments, and externally all alike, are distinguishable, after staining, into worker nymphs with a small vestigial frontal gland, and soldier nymphs with a large frontal gland.

7. The soldiers of *Eutermes pilifrons*, and also those of *E. morio* and *E. sanchezi*, are therefore not externally differentiated at the time of hatching, which, according to Bugnion, is the case in *E. lacustris*.

WELLESLEY, MASS.,
July, 1918.

BIBLIOGRAPHY.

Bugnion, E.

'12 *Eutermes lacustris* nov. sp. de Ceylan. Revue Suisse de zoologie, vol. 20.

'12b Obsérvations sur les termites. Différentiation des castes. Comp. Rend. soc. biol. Paris, I., t. 72.

Grassi, B., and Sandias, A.

'93–'94 Costituzione e sviluppo della società dei Termitidi. Atti Acad. Gioenia di sci. nat., Catania.

'96–'97 The Constitution and Development of the Society of Termites; Observations on their Habits; With Appendices on the Parasitic Protozoa and on the Embiidæ, translated by T. H. Blandford. Quart. Jour. Micros. Sci., vols. 39–40.

Knower, H. McE.

'94 Origin of the "Nasutus" (soldier) of *Eutermes*. Johns Hopkins Univ. Bull., vol. 13, pp. 58–59.

Snyder, T. E.

'15 Biology of the Termites of the Eastern United States with Preventive and Remedial Measures. U. S. Dept. Agr., Bur. Ent., Bull. no. 94.

Thompson, C. B.

'16 The Brain and the Frontal Gland of the Castes of the "White Ant," *Leucotermes flavipes* Kollar. Jour. Comp. Neurol., vol. 26.

'17 Origin of the Castes of the Common Termite, *Leucotermes flavipes* Kol. Jour. Morph., vol. 30.

RESEARCHES ON THE SEX-CHROMOSOMES OF PSYCHIDÆ (LEPIDOPTERA).

J. SEILER.

WITH ONE TEXT-FIGURE AND ONE PLATE.

The demonstration which I have given of the digametic condition of the female sex in Lepidoptera in the case of *Phragmatobia*,[1] gave a brilliant confirmation of the assumption made by genetic experimenters (Doncaster, Goldschmidt) that in butterflies the female sex must be digametic. In all other cases the digametic condition has been conclusively demonstrated only in the male, and as was to be expected, therefore, my results were doubted. For this reason I sought for a typical case in which the distribution of the sex-chromosomes could readily be demonstrated, and to work it out in such a manner as to leave no room for further doubt.

Such a typical case was found in certain species of Psychidæ. I was led to examine this group because the sex-ratios (which under certain conditions are quite atypical) gave reason to look for interesting cytological conditions; for it might be expected that in the parthenogenetic species, as is the case in *Angiostomum*, *Phylloxera*, etc., the preponderance or exclusive appearance of only one sex might be determined by the sex-chromosome mechanism. Unfortunately this point could not be demonstrated; on the other hand, the diœcious forms, *Talæporia tubulosa* and *Fumea casta*, were found to offer material suitable for my main purpose.

1. *Talæporia tubulosa* RETZ.

(*a*) *Maturation of the Egg.*—As the daughter-chromosomes of the first maturation-division separate, one chromosome remains at the middle of the spindle without showing at first any indication as to whether it will pass to the inner or the outer pole (Figs. 1–3). In Fig. 3 it will probably pass inwards, as may be

[1] '13, '14, *Arch. Zellforsch.*, Vol. XIII.

inferred from its relation to the slightly developed spindle-thickening. As the daughter-chromosomes move towards the spindle-poles the X-chromosome is found to lag behind, in some cases towards the outer plate, in others towards the inner. In Fig. 4 it is seen wandering inwards, in 5 outwards, in 6 and 7 inwards, in 8 outwards. During the interkinesis it is only occasionally recognizable (Fig. 9). In the metaphase of the second division (Fig. 10) it has without exception overtaken the autosomes and thenceforwards shows precisely the behavior of an autosome. Only in exceptional cases does the X-chromosome move from the beginning at the same rate with the autosomes; in such cases, of course, it is not distinguishable in side-views of the spindle.

In the Psychidæ, as in other cases (see Seiler, '14), chromatin is in some instances eliminated in large quantities during the first maturation-division, in others not at all.

The equatorial plate of the first maturation-division in the egg possesses 30 chromosomes, of which 29 are bivalent and one univalent. As is to be expected, the daughter-plates have different numbers of chromosomes. If the outer plate has 30 chromosomes the inner has 29, and *vice versa*. I have tried to obtain photographic proof of this, but the task is extremely difficult and tedious. Each egg contains but one spindle, and this must be so cut that the knife passes between the two daughter-plates without injuring them. Further, all the chromosomes (of course in both plates) must lie exactly in one plane and at right angles to the optical axis. Fig. 18 shows such an ideal plate with 29 chromosomes, and this photograph alone reproduces the size-relations of the chromosomes exactly as in nature. The sister-plate is not suitable for photographing. *All the plates photographed for this work are in themselves quite as clear and demonstrative* as Fig. 18. Since, however, the chromosomes did not always lie in the same plane, it was necessary to turn the fine adjustment somewhat during the exposure; hence the somewhat defective and in part ambiguous character of the figures. Figs. 11 and 12 are two sister-plates (in each case the first number designates the outer plate, the second number the inner), in the anaphase of the first division. Since the chromosomes undergo little or no change of relative position during the separation

of the daughter-plates, the homologous pairs can be identified with considerable certainty. In Figs. 13 and 14, again showing two sister-plates, the X-chromosome lies centrally.

Table I., which follows, gives a summary of selected cases in which both daughter-plates lie entirely in the same section, and which in other respects fulfill all the conditions of demonstrative clearness. Table II. summarizes the number of observed cases in which the X-chromosome as seen in lateral views of the spindle, is passing towards the outer or the inner pole. The result permits us to predict the primary sex-ratio to be expected in *Talæporia*. Those X-chromosomes that pass outwards enter the polar body, those that pass inwards divide equally in the second maturation-division and enter the female pro-nucleus.

TABLE I.

Locality.	No.	No. of Preparation.	No. of Chromosomes.	
			Outer Plate.	Inner Plate.
Breslau	1	1.14	30	29
"	2	1.10	29	30
"	3	26.6	30	
"	4	51.2	30	
Tornow	5	122.5	30	29
"	6	125.9	29	
"	7	136.4	30	29
"	8	136.1		30
"	9	136.9		29
"	10	136.10	29	
"	11	136.13	29	
"	12	136.17	29	30
"	13	137.3	30	29
"	14	138.5	30	29
"	15	138.7		29
"	16	138.8	29	
"	17	138.12		30
"	18	138.11		29
"	19	138.16	30	
"	20	139.11		29
"	21	139.13		29
"	22	140.6	29	30
"	23	140.10	29	
"	24	140.5	29	
"	25	149.7	29	30
"	26	150.20	30	
"	27	151.2		30
"	28	151.3	29	30
"	29	152.2	30	29
"	30	153.14	30	
"	31	153.16		30
"	32	155.1	30	
"	33	6.11	29	29
"	34	140.18	29	29

(*b*) *Maturation of the Sperm.*—In the spermatogenesis no sex-chromosomes can be distinguished. The equatorial plates of the first maturation-division show 30 chromosomes (Figs. 26, 27) and those of the second division the same (Fig. 28).

TABLE II.

Locality.	The X-Chromosome Passes		Total.	Sex Ratio.	
	Outwards.	Inwards.		Female.	Male.
Breslau.....	73	44	117	1.66	1
Tornow.....	61	45	106	1.35	1
	134	89	223	1.50	1

(*c*) *The Somatic Chromosome-number.*—Since two classes of eggs are formed, having respectively 29 and 30 chromosomes, we may expect to find two kinds of embryos, one having 59 chromosomes, the other 60. The actual conditions correspond with this expectation. In the stage of blastoderm-formation it is possible to count the somatic chromosome-number with absolute certainty. Figs. 15 and 16 show 60 chromosomes, though not so clearly as the actual object. Table III. gives a summary of the observed cases. Only such embryos were recorded as showed at least 2 (often 4 to 6) perfectly clear equatorial plates, each of which lay entirely in a single section and in the middle of its thickness, and in which no doubt could exist concerning the limits of a single chromosome.

TABLE III.

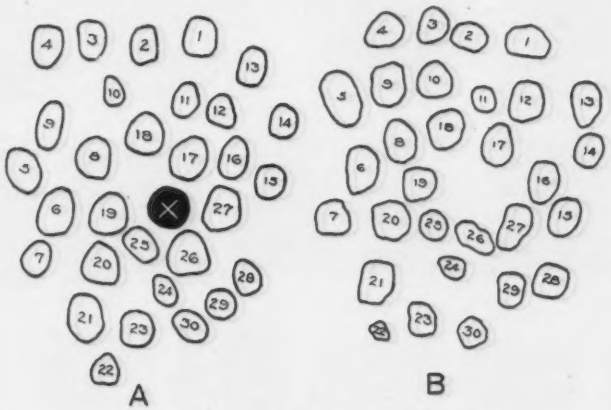
Locality.	Embryos.	No. of Chromosomes.
Tornow.....	30	59
"	25	60
"	4	58

As the table shows, a third sort of embryo could be demonstrated possessing 58 chromosomes. This determination can not possibly rest on an error, for in the first embryo seven perfectly clear plates all showed 58 chromosomes, in the second 6, in the third 5, and in fourth 2. Such embryos would probably produce but one kind of egg, containing 29 chromosomes. In

point of fact, I have two perfect pairs of daughter-plates, each with 29 chromosomes (see Table I., nos. 33, 34). No X-chromosome is present in these cases. If we leave these out of account there remain 35 females and 25 males, a sex-ratio of 1.36: 1 in favor of the female, that is to say, exactly the ratio to be expected from observations on the maturation-phenomena in the Tornow race. Possibly the embryos with 58 chromosomes may have arisen by the development of unfertilized eggs.

2. *Fumea casta* PALL.

The equatorial plate of the first maturation-division in the egg shows 31 chromosomes. One of these must be univalent, for the daughter-plates show in some cases 31 chromosomes, in others 30. As in *Talæporia*, an X-chromosome is present but strange to say it fails to lag behind the autosomes and passes together with them to one spindle-pole. Fig. 23, from a perfect plate with 30 chromosomes, clearly shows the natural size-relations. Figs. 19 and 20, 21 and 22, 24 and 25 are three pairs of daughter-plates, from the interkinesis, in which the chromosomes are perfectly clear and well-separated; but unfortunately the plates lay obliquely, so that the photographs seem to show certain fusions which do not really exist. Text-figure 1 shows the same



TEXT FIG. 1.

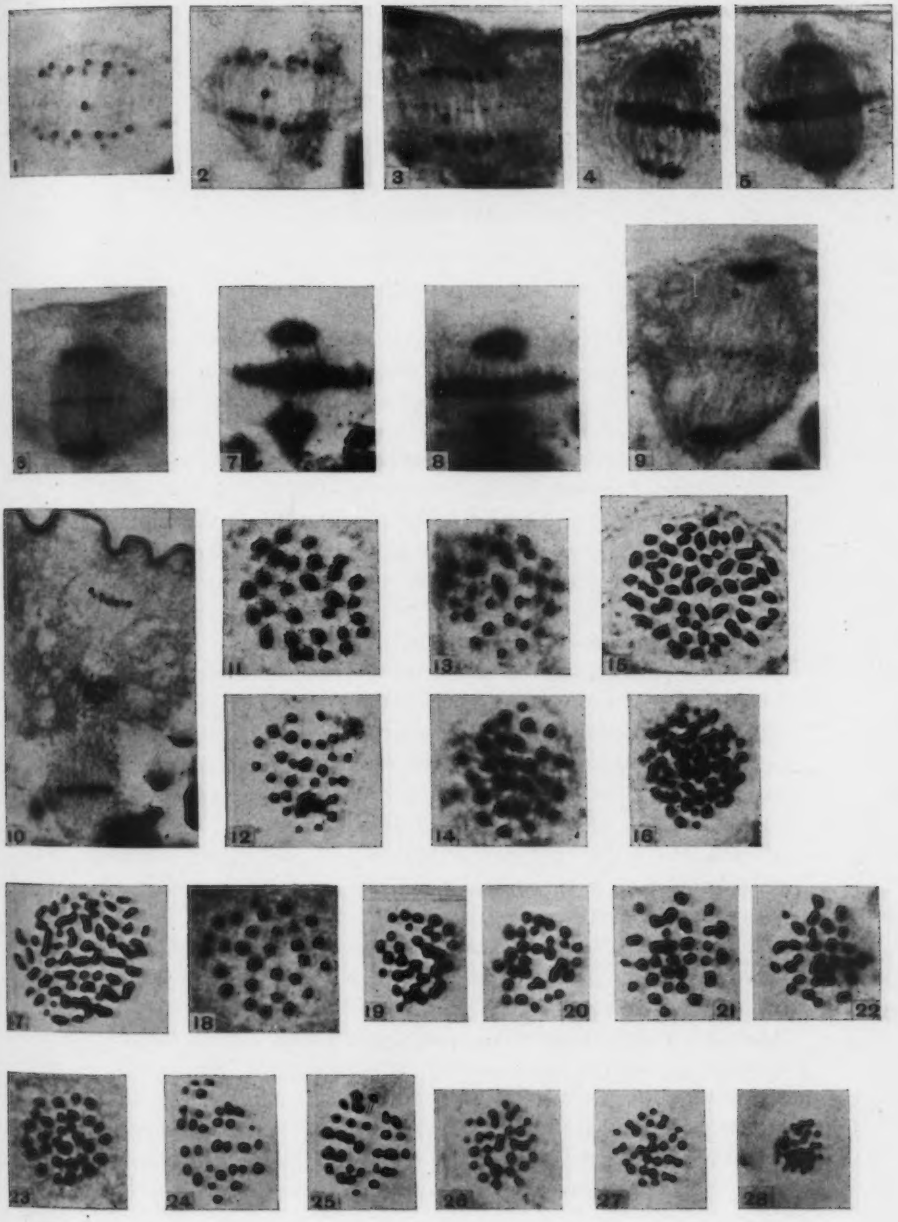
plates as Photos. 19–20, reproduced as accurately as possible in respect to the size-relations and relative positions, with an attempt to identify the homologous chromosomes. The X-chromosome happens, quite by accident, to be in all three cases in the outer plate; quite as often it is found in the inner one.

The embryos have either 61 or 62 chromosomes.

January, 1917.

EXPLANATION OF PLATE.

None of the photographs retouched. Enlargement 2,000, excepting Fig. 10, which is 1,000.



J. SEILER

THE RELATION OF THE PITUITARY AND THYROID GLANDS OF BUFO AND RANA TO IODINE AND METAMORPHOSIS.[1]

BENNET M. ALLEN.

UNIVERSITY OF KANSAS.

In the spring of 1918, Mr. Swingle and Mr. Etzen, working in our laboratory, discovered the fact that iodine administered in loose combination with flour brings about precocious metamorphosis in tadpoles after the same manner as the feeding of thyroid gland preparations previously demonstrated by Gudernatsch, '12. It at once occurred to the writer that iodine administration would afford a means of testing out the rôle of these two glands in metamorphosis. My plan was to administer iodine in this manner to tadpoles deprived of their thyroid glands, to those deprived of the pituitary gland and to those deprived of both. Since the thyroid gland has always been so completely identified with iodine in the animal body, the writer suggested that Mr. Swingle make it a part of his problem to feed iodine to thyroidless tadpoles. This he did with the result that he demonstrated the fact that the administration of iodine by feeding produces metamorphosis just as truly in thyroidless tadpoles as in normal tadpoles. It was shown by the work of Adler, '14, Smith, '16, and Allen, '16, that the removal of the pituitary gland of tadpoles has a marked effect upon the thyroid gland in that the colloid of the latter is of looser texture and smaller amount than normal. Associated with this is the fact that pituitaryless tadpoles do not undergo metamorphosis. A pituitaryless tadpole of *Rana pipiens* has been kept by the writer for two years without undergoing metamorphosis although it has now grown beyond the size normally attained by tadpoles of this species. These facts have led the writer to form the hypothesis that the pituitary gland may play an active rôle in metamorphosis, and that the thyroid gland would play the part of a storage organ. This view was expressed in an earlier paper

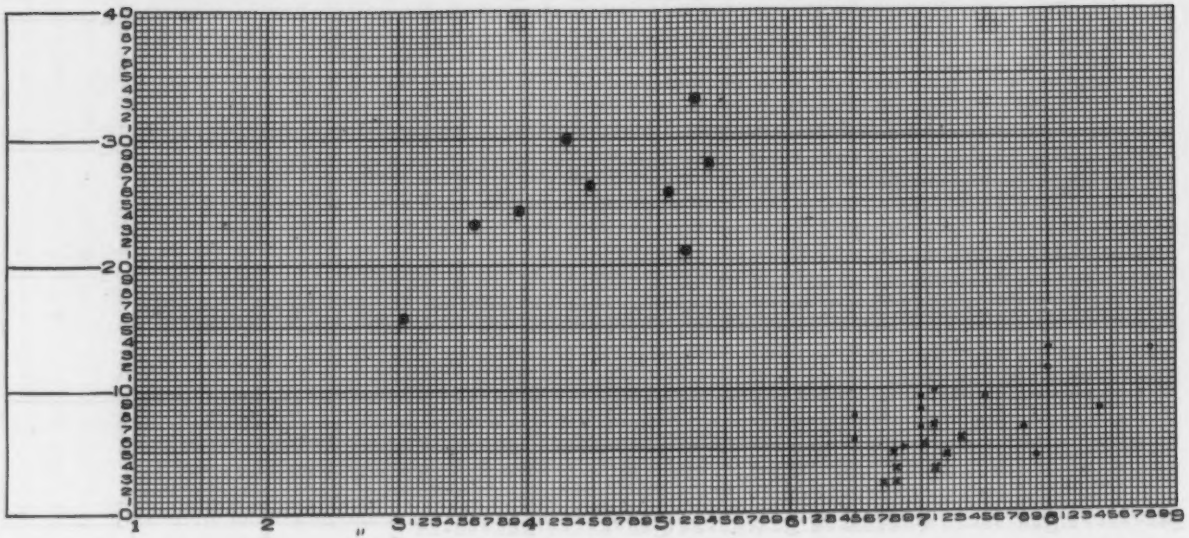


FIG. 1. Showing changes of body length and leg length of pituitaryless *Bufo* tadpoles to which iodine was fed. Abscissæ indicate body length in millimeters. Ordinates indicate hind leg length in millimeters. Dots indicate normal control tadpoles. Crosses indicate pituitaryless tadpoles at beginning of iodine feeding. Crosses in circles indicate pituitaryless tadpoles at end of iodine feeding.

(Allen, '17). Swingle's experiments showed that the administration of iodine alone without the mediation of the thyroid gland is able to produce metamorphosis. The peculiar correlation between the thyroid and pituitary glands made it seem highly desirable to carry on experiments in feeding iodine to tadpoles deprived of the pituitary gland and also to those deprived of both the thyroid and the pituitary glands.

The results of this experiment are so definite that they seem well worth recording. A large amount of caution is essential in work of this kind because it has been found that the characteristic color change described by Smith and Allen is not an altogether reliable test of the successful removal of the pituitary gland nor is the failure to metamorphose always to be relied upon as a test of success in the removal of either the pituitary or the thyroid gland. In certain cases, the color change has taken place in spite of the presence of a small remnant of the pituitary gland, and very greatly delayed metamorphosis has been found to take place in tadpoles in which a small fragment of the thyroid gland remained after the operation for removal. Notes along these lines have been published by the writer. For these reasons, it seemed best to make an intensive study of a limited number of specimens rather than to make a necessarily cursory study of a larger number. If the thyroid gland had been removed, the region where it should occur was sectioned and painstakingly studied in order to determine whether the operation had been successful. In the majority of cases where the pituitary gland had been removed, the region involved was likewise sectioned and examined for vestiges of the gland. The work was in this manner checked up to such a degree that the results are offered with full confidence of their accuracy. In these experiments, iodine was mixed with flour in the proportion of 1 to 100. Water was then added to make a creamy paste. This was then dried and the flakes were fed to the tadpoles. In the first series of experiments, *Bufo* tadpoles deprived of their pituitary glands were fed iodine for from ten to twenty-one days. This produced most striking results as shown in Tables I. and II. In this experiment, the tadpoles were kept in the same receptacle and as a consequence it was not possible to follow the changes in

individuals but we must consider mass results. There is a marked decrease in the size of the body. This is out of all proportion to the reduction in body size during normal metamorphosis. There is marked decrease in the length of the tail amounting to complete disappearance in one specimen which was unfortunately not preserved owing to the drying up of the shallow water in which it was necessary to keep it. In ten of the fourteen cases here recorded, both fore limbs had broken through the skin and in three of them the left one alone had appeared.

The difficulties of rearing these larvæ to complete metamorphosis are considerable, but not insurmountable. The small size of the larvæ is no doubt one factor. The sudden marked shrinkage of the body is a decidedly abnormal feature. These results are quite comparable to those attained by Swingle in normal and in thyroidless tadpoles. The removal of the pituitary gland in *Bufo* causes it to assume a light buff color in place of the familiar intense black. This color change was in no wise modified by the subsequent iodine feeding. Fig. 1 shows in graphic fashion the nature of these changes as regards the typical feature of hind leg length. The abscissæ represent the length of body while

TABLE I.

Bufo Pituitaryless Tadpoles Before Feeding Iodine.			No.	Iodine Feeding Begun May 31, 1918, After Feeding Iodine.					Days Fed.
Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.		Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Fore Leg Out.	
14.8	6.9	.50	1.	9.3	3.93	5.37	2.44	L.	21
14.6	7.2	.46	2.	5.9	3.60	2.34	2.34	R. L.	21
12.6	6.8	.22	3.	8.2	4.29	3.91	3.00	L.	28
13.0	6.7	.32	4.	9.2	4.49	4.71	2.67	R. L.	18
14.2	7.1	.76	5.	9.6	5.08	4.52	2.57	R. L.	18
16.1	7.3	.59	6.	7.5	3.03	4.47	1.52	R. L.	17
14.5	6.8	.46	7.	9.9	5.38	4.52	2.80	R. L.	18
14.4	7.1	.34	8.	11.3	5.28	6.02	3.30	R. L.	18
13.9	6.8	.31	9.	11.6	5.20	6.40	2.10	....	10
15.1	7.0	.54							
14.3	6.97	.44		9.18	4.67	4.69	2.59		

the ordinates represent the length of the hind legs. The location of any point serves to indicate the length of body by its horizontal location and the length of the hind legs by its height above

the base line. Dots are used to designate control, normal tadpoles; crosses designate the pituitaryless tadpoles just before the administration of iodine while crosses within circles indicate the pituitaryless tadpoles at the end of the period of iodine feeding. It appears that the pituitaryless tadpoles at the beginning of the experiment are at this stage already commencing to undergo a retardation in hind leg growth as compared with the normal controls. In the iodine fed, pituitaryless tadpoles the reduction in body length together with an actual and relative increase in leg length are clearly evident.

The next series of experiments involved feeding iodine in the same manner to tadpoles from which both the pituitary and thyroid glands had been removed. In this case, tadpoles were kept separated in different dishes so that it was possible to follow the growth of each individual tadpole. Unfortunately, all died within ten days. This may have been partly due to the excessively warm weather at the time. Because of the short period

TABLE II.

Bufo Pituitaryless Tadpoles Before Feeding Iodine.				No.	Iodine Feeding Begun June 18, After Feeding Iodine.					Days Fed.
Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.		Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Fore Leg Out.	
20.6	9.8	10.8	.93	10.	10.7	6.4	4.30	3.17	R. L.	18
18.8	9.7	9.1	.88	11.	9.1	4.55	4.55	2.05	L.	18
18.5	8.8	9.7	.51	12.	10.3	4.82	5.48	2.41	R. L.	23
15.6	8.4	7.2	.53	13.	8.2	5.15	3.05	2.57	R. L.	R. L.
14.8	7.3	7.5	.38		9.6	5.23	4.34	2.55		
18.8	8.7	10.1	1.37							
17.8	8.8	9.1	.77							
					Iodine Feeding Begun June 3.					
				14.	9.3	6.34	2.96	3.60	R. L.	15

of the experiment, the results are not so striking as one might desire but they point in the same direction as the other experiments and are quite significant. Table III. gives the results and needs no further explanation. Table IV. gives the dimensions of *Bufo* tadpoles deprived of both thyroid and pituitary glands but maintained under normal food conditions. A comparison of the tables shows that these do not have hind legs of length at all comparable with those that were fed iodine, even though the

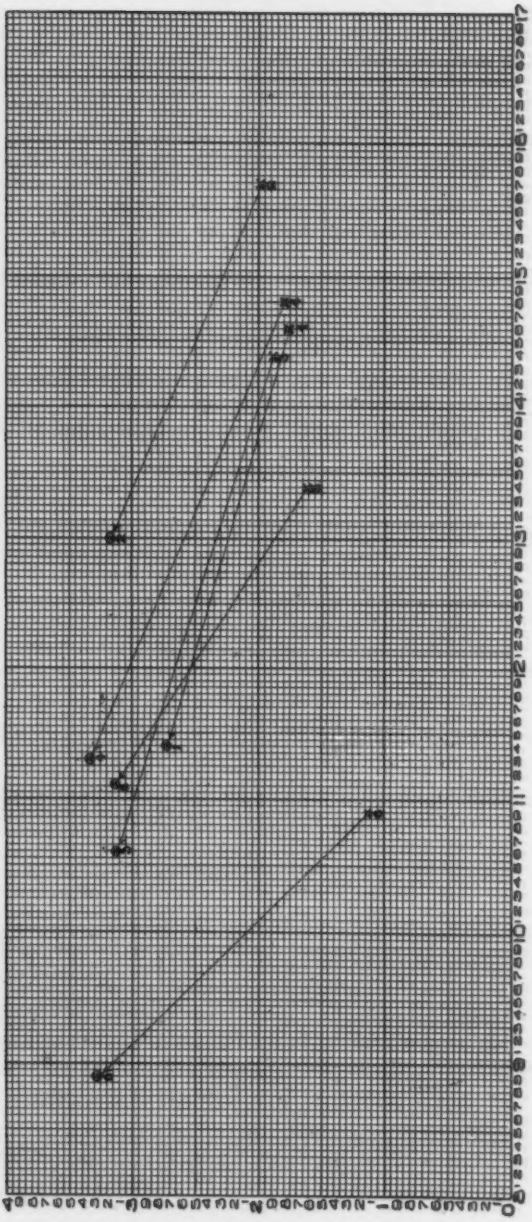


FIG. 2. Showing modifications of body length and leg length of pituitary-thyroidless tadpoles of *Bufo* to which iodine was fed. Abscissæ indicate body length in millimeters. Ordinates indicate hind leg length in millimeters. Arrows connect the points indicating any given individual at the beginning and the end of the experiment. The numbers of the points correspond to the numbers of Table III.

TABLE III.

Bufo PITUITARY-THYROIDLESS TADPOLES.

No.	Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.	Sucess of Operation.	Length of Feeding.
1.	33.1	14.6	18.5	1.75	No pituitary. No thyroid.	7 days.
2.	33.4	15.7	17.7	1.95	No pituitary. No htyroid.	10 days.
3.	32.5	14.4	18.1	1.91	No pituitary. No thyroid.	10 days.
4.	32.6	14.7	17.9	1.78	No pituitary. No thyroid.	10 days.
5.	29.1	13.4	15.7	1.62	Minute vestige of pituitary. No thyroid.	10 days.
6.	25.8	10.9	14.9	1.12	No pituitary. No thyroid.	10 days.
	31.2	13.9	15.5	1.69		

IODINE FEEDING BEGUN JULY 8.

Condition at End of Experiment.

No.	Total Length, Mm.	Body Length, Mm.	Tail Length, Mm.	Hind Leg Length, Mm.
1.	26.2	11.4	14.8	2.71
2.	25.1	13.0	12.1	3.17
3.	26.1	10.6	15.5	3.13
4.	27.2	11.3	15.9	3.33
5.	27.9	11.1	16.8	3.14
6.	18.5	8.9	9.6	3.27
	25.2	11.0	14.3	3.12

tadpoles represented in Table IV. were killed about two and one half months later and were then not only of larger size but older as well. Data are accumulating to show that the hind limbs continue to grow proportionately longer in older specimens than in younger ones of equal size. This seems to be generally true of thyroidless, of pituitaryless and of thyroid-pituitaryless tadpoles. More data upon this point must be gathered before a final conclusion can be reached because there are decided individual differences in the rate of limb growth in normal and operated tadpoles under apparently identical food conditions. In any case, a comparison of Tables III. and IV. will serve to answer any possible objections upon this score.

Fig. 2 shows the modifications of body length and leg length

indicated in Table III. The numbers of the points apply to the same individuals shown in the table. The arrow lines connect up points showing the dimensions of each specimen at the beginning and at the end of the feeding.

TABLE IV.

Bufo PITUITARY-THYROIDLESS TADPOLES.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Date Killed.
1.	20.4	9.8	1.75 K.	Sept. 24.
2.	23.0	10.9	2.14 K.	Sept. 24.
3.	24.1	11.3	2.54 K.	Sept. 26.
4.	26.1	11.4	2.41 K.	Sept. 24.
5.	25.4	13.2	1.91 K.	Oct. 31.
	23.8	11.2	2.15	

Table V. shows the results of feeding iodine to *Rana pipiens* tadpoles from which both the thyroid and pituitary glands had been removed. The experiment was begun with five specimens, two of which were not preserved. These five tadpoles at the beginning of iodine feeding had body lengths ranging from 10.3 mm. to 15.2 mm. and the hind legs ranged in length from .28 mm. in the smallest to .80 mm. in the largest. It is probable that no. 1 in Table V. represents the largest and no. 3, the smallest but it is not possible to be certain upon this point because the tadpoles were all kept together in the same aquarium. In any case, the growth of the hind legs is remarkable and it will be

TABLE V.

Rana pipiens WITH PITUITARY AND THYROID GLANDS REMOVED.
Fed Iodine from June 30 to July 25.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Fore Limbs.	Remarks.
1.	23.5	10.4	6.04	None	No thyroid. No pituitary.
2.	19.8	9.1	4.16	Left free	No thyroid. No pituitary.
3.	17.1	7.2	2.77	Left free	No thyroid. No pituitary.

noted that the left fore limb has appeared in nos. 2 and 3. The heads of these three tadpoles were sectioned and the series carefully studied in order to detect any remnants ever so small of thyroid or pituitary glands. Not the slightest vestiges of these

were found. In Table VI. the hind leg lengths of pituitary-thyroidless tadpoles normally fed and killed more than a month

TABLE VI.

Rana pipiens WITH PITUITARY AND THYROID GLANDS REMOVED.

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Date Killed.
1.	23.7	10.2	.43 K.	Aug. 22.
2.	28.3	11.2	.53 K.	Aug. 22.
3.	49.0	19.5	.76 K.	Oct. 7.
4.	59.8	23.3	2.28 K.	Aug. 21.

later than the above are found to be in sharp contrast to these. None of these pituitary-thyroidless *Rana pipiens* tadpoles were brought to metamorphosis by iodine feeding but there is little doubt but that it will be possible to do so; at any rate there is an extremely strong tendency in that direction.

TABLE VII.

SIZE OF THYROID GLANDS AND COLLOID MASSES OF PITUITARYLESS *Bufo* TADPOLES—IODINE FED. NUMBERS OF SPECIMENS CORRESPOND TO THOSE OF TABLES I. AND II.

Dimensions of Thyroid Gland.

No.	Total Length, Mm.	Body Length, Mm.	Side Length, Mm.	Width, Mm.	Thickness, Mm.	Volume, Cmm.	Aver. Vol., Cmm.	Aver. Diam. Colloid Mass, Mm.
1.	9.3	3.93	R. .100	.064	.031	.000198	.000209	.014
			L. .100	.071	.031	.000220		.014
3.	8.2	4.29	R. .137	.080	.037	.000405	.000409	.012
			L. .157	.080	.033	.000414		.014
7.	9.9	5.38	R. .149	.090	.036	.000482	.000364	
			L. 118.	.070	.030	.000247		
8.	11.3	5.28	R. .086	.070	.046	.000276	.000275	.024
			L. .078	.080	.044	.000274		.026
9.	11.6	5.20	R. .146	.090	.029	.000381	.000396	.016
			L. .089	.140	.033	.000411		.014
12.	10.3	4.82	R. .113	.070	.027	.000213	.000444	.014
			L. .157	.100	.043	.000675		.020
13.	8.2	5.15	R. .193	.100	.043	.000710	.000547	
			L. .150	.080	.032	.000384		
14.	9.3	6.34	R. .190	.121	.040	.000899	.001324	.016
			L. .220	.150	.053	.001749		.016
	9.8	5.04					.000471	.016

The iodine-fed pituitaryless *Bufo* tadpoles shown in tables I. and II. were sectioned and their thyroid glands studied, with the object of seeing whether the iodine feeding had brought about

any notable increase of colloid in the thyroid gland. Two were preserved intact for demonstration purposes. Only eight out of the twelve tadpoles sectioned were found to be in a satisfactory state of preservation for histological study. This was due to the fact that the tadpoles were usually preserved after they had died. Although the aquaria were frequently examined, the summer heat often caused considerable disintegration of the thyroid gland before the specimens could be preserved. An examination of Table VII. shows the length, width and thickness

TABLE VIII.

SIZE OF THYROID GLANDS AND COLLOID MASSES OF PITUITARYLESS *Bufo* TADPOLES NORMAL FEEDING AS CONTROLS:

No.	Total Length, Mm.	Body Length, Mm.	Hind Leg Length, Mm.	Side Length, Mm.	Breadth, Mm.	Thickness, Mm.	Volume, Cmm.	Average Volume, Cmm.	Diameter of Colloid Masses, Mm.
1.	16.7	7.4	.92	R. .110	.117	.047	.000604	.000469	.013
				L. .130	.081	.069	.000335		
2.	13.7	6.6	.36	R. .060	.150	.076	.000684	.000523	.011
				L. .070	.089	.060	.000373		.010
3.	17.8	8.7	1.09	R. .130	.111	.057	.000822	.000791	.011
				L. .150	.118	.043	.000761		.016
4.	13.6	6.4	.40	R. .090	.110	.078	.000722	.000679	.011
				L. .090	.107	.061	.000587		.011
5.	14.0	6.7	.40	R. .070	.080	.036	.000201	.000230	.013
				L. .090	.072	.040	.000259		.013
6.	14.0	7.0	.56	R. .180	.094	.041	.000693	.000629	.014
				L. .160	.104	.034	.000565		.014
Aver	14.6	7.1						.000554	.013
7.	22.5	10.5	3.23	R. .330	.193	.057	.003330	.003382	.024
				L. .339	.153	.068	.003435		.024
8.	26.8	13.4	4.16	R. .460	.236	.057	.006187	.006770	.028
				L. .490	.224	.167	.007353		

of each thyroid gland. The specimens are designated the same as in Table I. From the dimensions of each gland was calculated the volume of a parallelopiped that would contain it. This of course would constitute but a rough relative approximation to the volume. Table VIII. shows similar measurements and calculations made upon the thyroid glands of normally fed pituitaryless *Bufo* tadpoles of size corresponding to these as measured at the beginning of iodine feeding. A comparison of Tables VII. and VIII. will show that the thyroid glands taken as

a whole have shrunken appreciably together with the shrinkage of the body as a whole; but not proportionately as much. In the iodine-fed tadpoles the colloid masses show a slightly greater average diameter than do those of the normally fed specimens. These facts do not seem especially significant. The slight increase of the colloid masses and the actual decrease in the dimensions of the gland roughly counterbalance one another. It is to be admitted that the number of specimens is small but it is large enough to demonstrate that no striking changes in the size of the thyroid glands and of their colloid content in pituitaryless tadpoles result from iodine feeding, at least, not within the stages and time limits of the experiment. It will be interesting in future experiments to test whether feeding iodine at early stages prior to the normal time of colloid formation would cause it to appear precociously.

In a paper upon the "Development of the Thyroid Glands of *Bufo* and their Normal Relation to Metamorphosis," Allen (in press), a study was made of the relation of the growth of the thyroid gland to the growth and metamorphosis of *Bufo* tadpoles. The stages used are a trifle more advanced than those used in this experiment. It appears, however, that the thyroid glands are of about the same size in their early stages in pituitaryless tadpoles as in normal tadpoles and that the colloid begins to form at the same time in both.

Summary and Conclusions.

At the beginning of this paper, attention was called to the fact that the growth of the thyroid glands and the accumulation of colloid in them are retarded by the removal of the pituitary gland. Rogers and Larson have both shown that the removal of the thyroid gland causes an hypertrophy of the pituitary gland (Rogers, '18, anterior lobe; Larson, '19, anterior and intermediate lobes). It would be premature to offer an interpretation of these facts but they seem to justify the hypothesis that these glands are closely interrelated. The writer has for several years favored the hypothesis that the thyroid gland may, largely at least, play the rôle of a storage organ for the iodine of the body, at the same time controlling its distribution. It even seems

possible that the pituitary gland might play the chief rôle in the utilization of iodine by the body. This view is in the main based upon the fact that tadpoles of *Rana* and *Bufo*, deprived of the pituitary gland, fail to metamorphose in spite of the fact that the thyroid glands remain intact and even though the body of the tadpole reaches gigantic size. The removal of the pituitary gland renders the thyroid gland as powerless to bring about metamorphosis as though the thyroid gland were itself removed.

It was shown by Swingle that metamorphosis could be produced in normal tadpoles by feeding them iodine. He likewise showed that iodine feeding would produce metamorphosis even in tadpoles deprived of the thyroid glands. Simultaneously with the above work, the writer was carrying on the experiments that form the subject of the present paper. They show conclusively that metamorphosis can be produced by iodine feeding in tadpoles deprived of the pituitary glands. More striking still is the demonstration of the fact that feeding iodine to tadpoles deprived of both thyroid and pituitary glands can carry them far in the process of metamorphosis, the attainment of which was prevented only by the death of the tadpoles.

In the light of all these facts, we are justified in drawing the following general conclusions:

With normal feeding, involving the intake of very minute quantities of iodine, the presence of both the thyroid and pituitary glands is essential to enable the animal to undergo metamorphosis. Unpublished data show quite conclusively that *Rana* and *Bufo* larvæ proceed to the same stages of limb growth in the absence of the thyroid or of the pituitary gland or of both together. The administration of large quantities of iodine will cause the tadpoles to undergo metamorphosis in the total absence of the thyroid gland or of the pituitary gland or in the simultaneous absence of both thyroid and pituitary glands. It may be found by later experimentation that iodine is not the only factor that is capable of bringing animals to maturity. Investigation along these lines is still in its infancy. Before this paper appears in print, the writer expects to have under way more extensive quantitative experiments that will determine the reactions of normal, pituitaryless, thyroidless, and pituitary-

thyroidless tadpoles to these and to other factors that influence development.

LITERATURE.

Adler, L.

'14 Metamorphosestudien an Batrachierlarven. I., Extirpation endocriner Drusen. II., Extirpation der Hypophyse. Arch. f. Entwickelungsmech., Bd. 39.

Allen, B. M.

'16 Extirpation Experiments in *Rana pipiens* Larvæ. Science, November 24, 1916.

Allen, B. M.

'16 Effects of the Extirpation of the Anterior Lobe of the Hypophysis of *Rana pipiens*. BIOL. BULL., Vol. 32, March, 1917.

Allen, B. M.

'16 The Results of Thyroid Removal in the Larvæ of *Rana pipiens*. Jour. Exper. Zool., Vol. 24, p. 499, January 5, 1918.

Gudernatsch, J. F.

'12 Feeding Experiments on Tadpoles. Arch. f. Entwickelungsmech., Vol. 35, p. 457.

Gudernatsch, J. F.

'14 A Further Contribution to the Knowledge of Organs with Internal Secretion. Amer. Journ. Anat., Vol. 15, p. 431.

Larson, M. E.

'19 Effect of the Extirpation of the Thyroid Gland upon the Pituitary Gland in *Bufo*. Anat. Record, Vol. 15, p. 353.

Rogers, James B.

'18 The Effect of the Extirpation of the Thyroid upon the Thymus and the Pituitary Glands of *Rana pipiens*. Jour. Exper. Zoöl., Vol. 24, p. 589, January 5, 1918.

Smith, P. E.

'16 Experimental Ablation of the Hypophysis in the Frog Embryo. Science, August 25, 1916.

Smith, P. E.

'16 The Effect of Hypophysectomy in the Early Embryo upon the Growth and Development of the Frog. Anat. Record, October, 1916.

Swingle, W. W.

'19 Studies on the Relation of Iodine to the Thyroid. I. The Effects of Feeding Iodine to Normal and Thyroidectomized Tadpoles. Jour. Exper. Zoöl., Vol. 27, p. 397, January 5, 1919.

Swingle, W. W.

'19 Studies on the Relation of Iodin to the Thyroid. II., Comparison of the Thyroid Glands of Iodin-fed and Normal Frog Larvæ. Jour. Exper. Zoöl., Vol. 27, p. 417, January 5, 1919.

THE MIGRATION OF REPRODUCTIVE ORGANS FROM PARENT TO BUDS IN HYDRA.

GEORGE W. TANNREUTHER,

ZOÖLOGICAL LABORATORY, UNIVERSITY OF MISSOURI.

The germ cells of hydra, as far as we are able to interpret by means of a microscopic study of carefully prepared sections, originate directly from the ordinary interstitial cells. It is true, however, that some investigators advocate the specificity of the germ cells. But the species studied gave no evidence of a specific line of germ cells. It is not my intention to take up a careful study of the germ cells in hydra, as to their method of origin and history, but rather to consider them from the standpoint of their place of origin. That is, do the reproductive organs originate directly on the forming buds, or are they formed on the parent hydra and later migrate to the buds during their development.

Investigators have reported the presence of sex organs on the buds in hydra, but in no case has it been definitely stated that these organs originated directly from the ectoderm of the young buds on which they were found.

Fig. 1 represents a camera drawing of *Hydra vulgaris* in a partially contracted condition, with five buds in different stages of development, and thirteen spermaries. Most of the spermaries were completely formed before budding begun. In no instance did the spermaries originate directly on the buds, but migrated with the cells of the parent to those of the forming buds.

Diagram A, 1, represents a small portion of the parent hydra extended, with a bud in its initial stage of development. The area between *R-G* on either side of the bud, indicates the region of growth or the region that contributes directly to the formation of the bud. Reproductive organs found within this region (*R-G*), migrate from the parent hydra to the bud. Those outside of this region of growth never reach the bud, but in most instances retain their original position, while the buds

are developing. Those on the border line, often migrate towards the buds but never reach them. That is, they remain on the parent. The place occupied by the reproductive organs on the completely formed bud is determined by their position in the

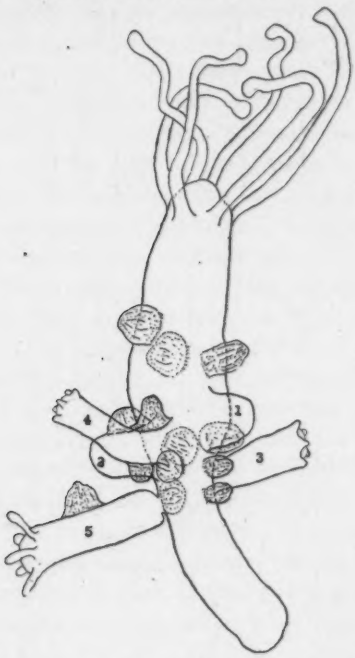
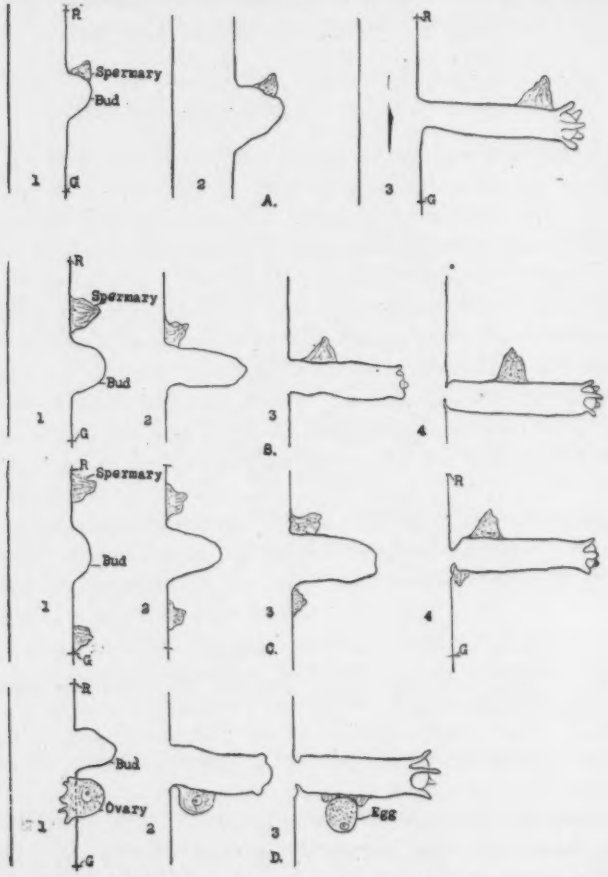


FIG. 1. Hydra with 5 buds and 13 spermaries. Spermaries have migrated from parent to buds 2, 4 and 5.

growth region (*R-G*) of the parent hydra at the time the bud starts.

Spermaries or ovaries found near the tip or center of the distal end of the bud when it makes its first appearance, take up a final position at the distal end of the completely formed bud (Diagram A, 3). The spermary (Diagram A, 1–3) during the different stages of progress in the development of the bud, retains its same position with reference to the tip or distal end. In Diagram A the bud became independent of the parent in sixty hours after its initial stage of development. Diagram B,

1, 2, 3 and 4 shows the position of the spermary at different periods of growth on the bud, and its final position when the bud is completely formed. Note the position of the spermary



DIAGRAMS A, B, C and D to show the migration of reproductive organs from parent hydra to the forming buds.

before its migration began. In Diagram C, 1, 2, 3 and 4 the spermaries were located near the outer edge of the region of growth (*R-G*). The time required for the development of the

bud in Diagram C was seventy hours. Diagram D, 1, 2 and 3 shows the position taken by the ovary, at the different stages in the development of the bud. The rate of growth and migration of the ovary is the same as in the case of the spermary.

The position taken by the reproductive organs during the different periods of growth in the formation of the buds, indicate beyond question, that the region around the base of the buds, contributes almost entirely to the formation and growth of the buds, and that the cells of the buds contribute but little to their own development. The cells in the growth region not only divide mitotically, but migrate from the parent hydra to the buds and carry the reproductive organs with them.

It was not an unusual thing to find hydra that were produced by budding in the different cultures, which showed either ovaries or spermaries at their extreme distal or proximal end. Diagrams A, 3, and C, 4, are good examples of these conditions.

In a few instances where the spermary was situated equidistant between two forming buds, the spermary became divided through the central region, either half reformed into a distinct spermary and migrated towards the corresponding bud. In these particular cases the spermaries were in their early stages of development. But in those spermaries that were completely formed and contained mature sperm, no division occurred. They either remained in their former position equidistant between the two buds, or migrated in toto towards one or the other.

The migration of the reproductive organs as observed in hydra is rather an interesting phenomenon, and occurs quite frequently, especially in budding hydra that are rich in sex organs. This process of migration however, is directly associated with asexual reproduction and can not be considered as having any very special significance, since the reproductive organs migrate in conjunction with the surrounding cells and not independently of them.

LITERATURE CITED.

Downing, E. R.

'04 The Spermatogenesis of *Hydra*. Zool. Jahr., Band 21.

Lang, Albert

'92 Über die Knospung bei *Hydra* und einigen Hydropolypen. Z. wiss. Zool., Vol. 54.

Tannreuther, G. W.

'09 Observations on the Germ Cells of *Hydra*. BIOL. BULLETIN, Vol. XVI.

'09 Budding in Hydra. BIOL. BULLETIN, Vol. XVI.

Wagner, R. E.

The Oögenesis and Early Development of *Hydra*. BIOL. BULLETIN, Vol. XVII.

COLUMBIA, MO.,
March 30. 1919.

RESISTANCE OF CILIA TO CYTOLYTIC AGENTS

M. E. COLLETT,

UNIVERSITY OF PENNSYLVANIA.

In studying the hæmolysis of erythrocytes from a series of mammals, Rywosch ('07) found that the order of resistance to saponin was almost the reverse of that to hypotonic NaCl. He did not however offer an explanation. Hoeber in 1912 suggested that saponin might affect the lipoids, but that the hypotonic solutions attacked some other component of the cell. K. Meyer (reviewed by Port) noted that corpuscles rich in cholesterol were also most resistant to saponin. The protective action of cholesterol in a cholesterol-lecithin mixture against saponin has been demonstrated on artificial membranes, and several workers (reviewed by Port, '10) very early cited Abderhalden's analyses of erythrocytes in support of the view that cholesterol is the chief factor in determining saponin resistance. Hoeber ('08 and '14) used several hypotonic salt solutions, and found that the phosphates were least injurious if used alone, but if mixed with saponin became the most injurious of the series. He interpreted this as due to the action of PO_4 on the cell colloids, but not until 1914 did he offer the following more elaborate theory. A cell protein which normally is stabilized by PO_4, will, after undergoing a change in sign (as by the action of acids), be destroyed by PO_4. Saponin produces such a change in sign of the corpuscles and therefore attacks especially cells rich in PO_4. This may explain the fact that a cell resistant to hypotonic NaCl is relatively susceptible to saponin.

The present experiments were undertaken to find whether this curious reversal of resistance occurs in cells of another sort (ciliated cells of various marine invertebrates), and to compare the effect of hypotonic NaCl with that of a hypotonic solution which is at the same time balanced, *e. g.*, dilute sea water.

The ciliated gill filaments of several molluscs and the ciliated larvæ of echinoderms, molluscs and annelid worms furnished

material for the experiments. The gills were divided into narrow strips of one or two filaments. The larvæ were generally heliotropic and so could easily be secured in large numbers in very little water. Not all the larvæ were fully normal: *Echinarachnius*, *Arenicola* and *Asterias* were the most variable in vitality. All of the solutions used (except of course NaCl) were made up in sea water rather than in distilled water and were never kept more than eight hours: not over four hours in the case of volatile substances. The experiments were carried out in stoppered test tubes which for higher temperatures were floated in warm water. The temperature of the tubes was read during every experiment. Each day's results were recorded on a separate slip of paper. The summary given below is an average of all observations and where these vary because of temperature, difference in viability (or other experimental error), the order of susceptibility has been verified by reference to the separate experiments in which the conditions must have been the same. The results obtained are as follows:

In comparing the resistance to saponin with that to hypotonic NaCl it is clear that although there are some irregularities (which are starred), the general order is the same. This is quite unlike the sharp reversal of order obtained with erythrocytes in saponin and in hypotonic NaCl. The order of cilia resistance in

TABLE I.

Saponin (per cent. represents dilution of a stock solution of 0.5 per cent. in sea water).

	20–23° C.							
Per Cent.	.25.	.5.	1.	5.	10.	30.	50.	100.
Echinarachnius gastr.....	8	5	5	2				
Arbacia 1 d.....		10	4	< 4				
" 2 d.....		10	8		3			
" 3 d.....	20	10+	6		3			
Echinarachnius pluteus.....			11	4	2			
Arenicola 2 d.....			15	3	4	3		
Chætopterus 1, 2 d.....			22	6	5	3		
" 3 d.....			15+	16	8	4		
Nereis 1 d.....			40+	12	8	5	3	
" 2 d.....			45	19	9	8	4	3
" 3 d.....			50		8	8	6	
Asterias 1–10 d.....			30+	60				80+
Cumingia 1, 2 d.....			30+					30+

Hypotonic Sea Water. *Hypotonic NaCl* (stock solution).

Per Cent.	20–23° C. 40.	35.	30.	20.
Echinarachnius plut.	30	15	5	< 2
" " gastr.	<30	15	5	< 2
Arbacia 1 d.		20+	4½	2
Arbacia 2 d.				
" 3 d.			7	2
Arenicola 2 d.	30+		8	5
Chætopterus 1, 2, 3 d.			20	9
Nereis 2 d.	30+	20+	12–22	8
Asterias 1–10 d.			18	9
Cumingia			30	22

Per Cent	20–23° C. 40.	30.	20.
Arbacia	11	8	
Arenicola	25+	5	
Echinarachnius	15+	< 2	
*Asterias 6 d.	15	18	15
*Cumingia		25	15
Chætopterus	25	27	
Asterias 2, 7, 8 d.	7	25	10
Asterias 1 d.	15	30	15
Nereis		30	4

TABLE II.

Saponin (per cent. are presents dilution of a stock solution of 0.5 per cent. in sea water).

Per Cent	20° C. 10.	20.	30.	50.	100.
Mytilus			8	4	2
Pecten	15	15	12	10	4
Mya	20	20	10	8	8
Quahog	25+	20+	15	8	8
Razor clam	50	30	30	18	17
Modiola	50+	30	30	27	20

Hypotonic Sea-water. *Hypotonic NaCl.*

Per Cent	20° C. 20.	15.	10.	5.
Pecten	6	4		2
Mytilus	35	16	8	3
Mya	35	15	9	4
Quahog	40	20	14	4
Razor clam	45+	35	14	4
Modiola	45+	35	14	4

Per Cent	20° C. 15.	10.
Pecten	<3	<3
Mytilus	5	<3
Mya	5+	4
Quahog		7
Razor clam		8+
Modiola		

hypotonic sea water is almost exactly the same as in saponin solution: there is in fact only one slight difference (*Echinarachnius plut.*) as against two in NaCl. Evidently hypotonic sea water has not quite the same action on the cilia as hypotonic NaCl, but the difference is not great. No data on the hæmolytic power of a hypotonic balanced solution are available for comparison.

The action of acids upon erythrocytes (Koeppe, cited by Rywosch) is a function of their H^+ concentration, but their action upon cilia of protozoa (unpublished experiments) and of the giant clam (Harvey) is correlated with other factors as well.

Still another evidence of the peculiarity of erythrocytes is found in temperature coefficients. They show with a rise in temperature increased susceptibility to acids and to chloroform (Rywosch) but not to saponin or to hypotonic NaCl (Kagan). As the following table indicates this is not the case with most of the ciliated cells.

TABLE III.

Saponin.

	20–23° C.					25° C.			30° C.	
Per Cent.	0.5.	1.0.	30.	50.	100.	1.0.	30.	50.	0.5.	100.
Echinarachnius pl.		11				3				
Arbacia 2 d.	10	15	3			3			8	
Chætopterus 2 d.		22	3				3			
Nereis 1 d.		40	5	3				3		
Cumingia 2 d.		30			30			50		
Asterias 2 d.		60+			80			20		
" 3 d.								25		30
" 6–10 d.								60		

Hypotonic Sea Water.

	20–23° C.			25° C.		
Per Cent.	35.	30.	20.	35.	20.	20.
Echinarachnius pl.	15	5	2	10	3	2
" g.	15	5	2	20	5	
Arbacia 3 d.		7	2	25	5	2
Nereis 2 d.	20	15	8			13
Asterias 2 d.		18	9	60	15	9
" 4 d.				30	7	
" 6–10 d.					12	10

TABLE IV.

Saponin.

	20° C.					30° C.				
Per Cent.	10.	20.	30.	50.	100.	10.	20.	30.	50.	100.
Mytilus			8	4	2		4			
Pecten	15	15	12	10	4	5	4	2	2	
Mya	20	20	10	8	8		13	3	3½	
Quahog	25	20	15	8	8		16	5	4	
Razor clam	50	30	30	18	17	11	8	5	2½	3
Modiola	50	30	30	27	20	15	12	6	4½	4

Hypotonic Sea Water.

	20° C.				30° C.	
Per Cent.	20.	15.	10.	5.	20.	15.
Pecten	6	4		2	3	3
Mytilus	35	16	8	3	30	10
Mya	35	15	9	4	40	13

Thus resistance of cilia to both saponin and hypotonic NaCl is lowered by increasing temperature instead of remaining constant as is the case with erythrocytes. The coefficients are higher in saponin than in hypotonic sea water and also vary considerably from one form to another. This is not the case with erythrocytes. However the action of acids upon various infusoria, as upon erythrocytes, is markedly altered by change in temperature.

A few experiments were made using chloroform, ether and acetone made up in sea water (in vols. per cent., chloroform 0.1–0.2, and 0.37 per cent.; ether 2–6 per cent., acetone 10 per cent.). The gill cilia showed the same order of resistance in these reagents as in hypotonic sea water. The larval cilia were more irregular, as the following list shows, though the order is not very different from that obtaining in saponin.

Chloroform.	Ether.	Acetone.
Echinarachnius p.	*Echinarachnius* g. & p.	*Echinarachnius*
" g.	*Arbacia* 1, 3 d.	*Arbacia* 2, 3
Arbacia 1 d.	**Cumingia*	" 1
**Asterias* 3–4	*Asterias* 3, 6–10 d.	**Asterias* 2–6, 10
* " 2	*Chætopterus*	**Cumingia*
Arenicola 2	*Nereis* 1, 2	*Chætopterus*
Nereis 2	**Arenicola*	**Arenicola*
Cumingia	*Asterias* 1, 2	*Asterias*
Asterias 6–10		

With erythrocytes Rywosch found in each of these reagents a quite different order, more irregular than was observed in the present experiments.

An adequate explanation of these results is impossible at present. Although detailed analyses of corpuscles have long been available, there are analyses of only two of the organisms used in the present experiments, made upon the eggs (not larvæ) of *Arbacia* and *Asterias* by Matthews. He found the *Arbacia* egg rich in cholesterol as compared with lecithin and the *Asterias* egg the reverse. If these lipoids alone determined resistance to saponin and to hypotonic NaCl, *Arbacia* should be more resistant than *Asterias*, but this is not the case. This may be due to a change in the cholesterol content as the egg develops into a larva. Certainly differences due to age occur in most of the forms, especially at times of great morphological change. How-

ever, until we have adequate analyses we cannot offer a definite explanation of the characteristic differences in the behavior of these ciliated cells toward reagents.

Summary.

1. Cilia which are resistant to saponin are generally resistant to hypotonic sea water and hypotonic NaCl. The reversal of resistance found with erythrocytes does not hold with cilia.

2. Relative resistance of cilia to hypotonic NaCl is not the same as resistance to hypotonic sea water, although the irregularities are not numerous.

3. Most of the cilia show a change in resistance with change in temperature. This is more marked in saponin than in hypotonic sea water.

I am indebted to Dr. M. H. Jacobs for suggesting this problem and criticizing the results, and to Dr. C. E. McClung for the use of a table at the Marine Biological Laboratory at Woods Hole.

Bibliography.

Harvey
'14 Internat. Zeit. f. Ph-Chem. Biol., I.
Hoeber
'13 and '14 Physikalische Chemie d. Zelle, 2te u. 3te Aufl.
'08 Biochem. Zeit., 14.
'14 Biochem. Zeit., 60, p. 131.
Kanitz
Winterstein's Handb. d. vergleich. Physiol. (for analyses of corpuscles).
Kagan
'13 Folia Hæmatologica, 17, p. 211.
Matthews
'13 J. Biol. Chem., 14, p. 465.
Port
'10 Deutsch. Arch. klin. Med., 99, p. 259.
Rywosch
'07 Pflueger's Archiv, 116, p. 229.